Papa Spunta TICAR®

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PRESENTATION

Tecnoplant S.A. is an associate firm of the Sidus Group that, since 1999, has been developing, together with the National Council of Scientific and Technical Research (CONICET), a genetically modified potato resistant to the PVY virus (Potato Virus Y). Argentine authorities have recently approved its commercialization.

The PVY (see FIGURES 1a and 3) itself or together with another virus can produce losses for more than 40% in crop production (see FIGURE 2). Thus, potato producers in our country have to buy seeds every year so as to avoid such important productivity loss.

Potatoes are liable to an ample variety of pathogens (viruses, bacteria, fungi, etc.) that do not harm consumers but raise production and maintenance costs, and affect productivity. That is why Tecnoplant S.A. has developed **Spunta TICAR** as a solution to the PVY viral infection problem and, also, with the idea of lowering the use of pesticides in production fields, which are used to control the aphids that transmit the virus.

Potatoes multiply through clones, which are either pathogen free or have low pathogen levels. The presence of PVY infected plants in the potato production fields for fresh consumption and the incidence of aphids in the virus transmission (see FIGURE 1b) make that potatoes planted during a campaign be completely replaced in the following one. So, producers cannot keep the seed from one campaign to the next one if they aim to obtain good yields. This obliges producers to buy disease-free seeds every year.

This seed must grow in isolated areas within the fields in which potatoes are cultivated for fresh consumption (this is a way to avoid the virus transmission through the aphids) and must also come from healthy crops. The cultivation of disease-free seeds starts in in-vitro laboratories from cleaned-up plants developed from meristems. Next, these seedlings are taken to isolation greenhouses to produce microtubers, with which field multiplication in isolated areas starts and increases yearly. This complex system, which approximately takes a 5-year investment, raises the production costs of the seed and, in turn, its price.

Tecnoplant S.A., through the development of **Spunta TICAR**, provides a cheap and eco-friendly solution to potato production for fresh consumption in Argentina. This technology can potentially be taken to other countries.

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Potato virus

LThe diseases caused by the virus affect the whole potato plant. They are transmitted vegetatively (through the use of infected seed tubers), mechanically (through contact between leaves, workers and machinery) and, mainly, through vector aphids (insects that transmit the virus) (see FIGURE 1 (b)). There are not antiviral products to control vegetable viruses. The only means to control them are the use of virus-free seeds and pesticides to eliminate the aphids (vectors).

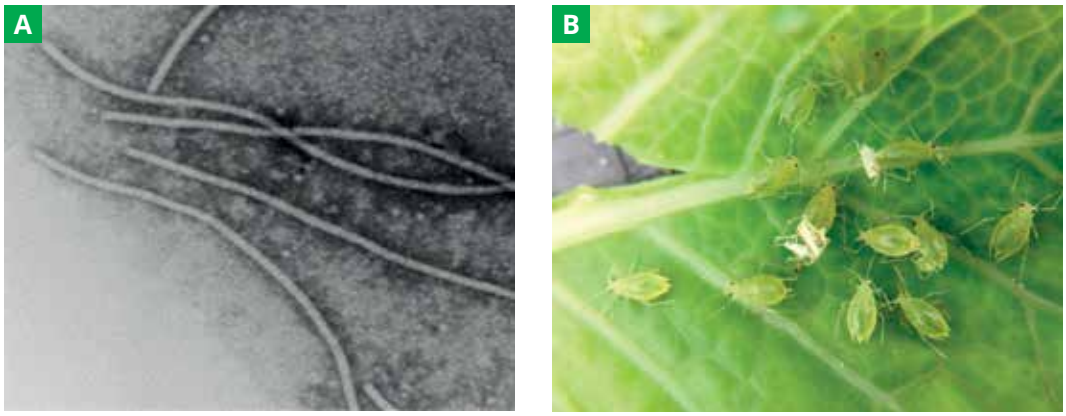

FIGURE 1: Electron microscopy images particles of the PVY virus (a) Vector aphids (b).

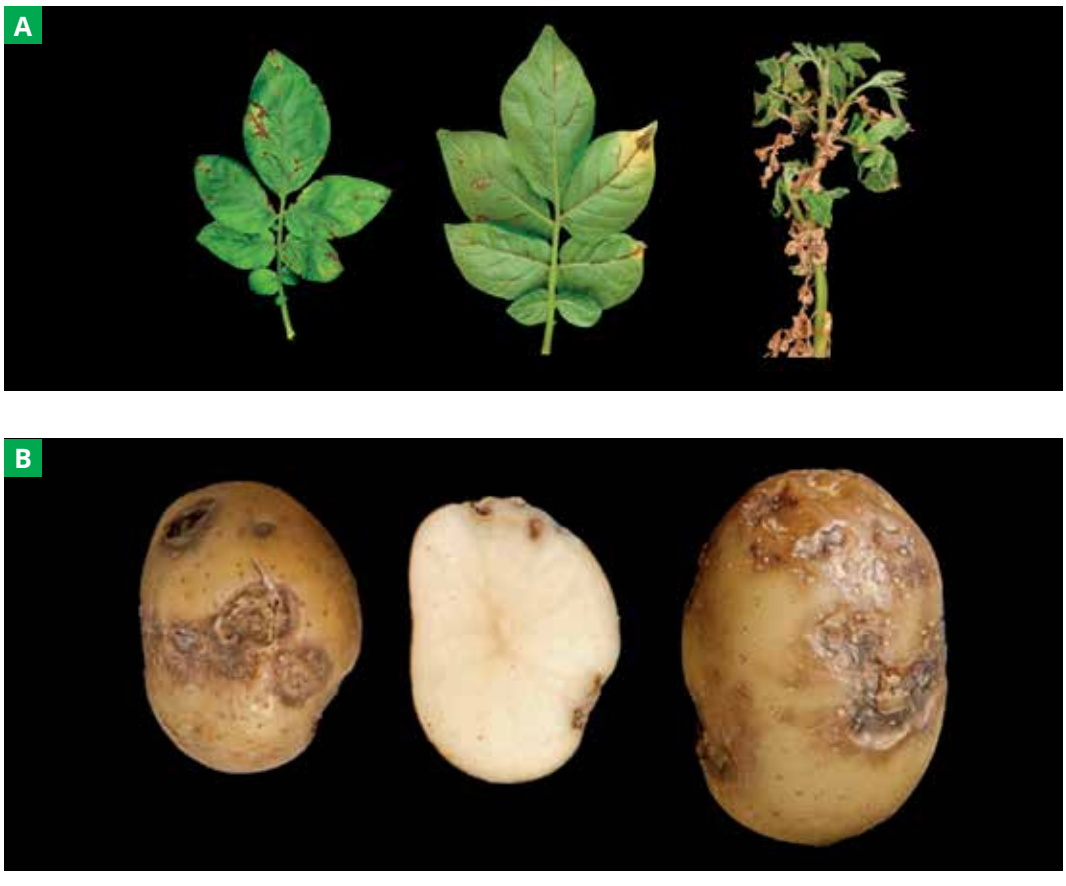

FIGURE 2: symptoms of PVY infection on leaves (a) and tubers (b).

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Generic improvement

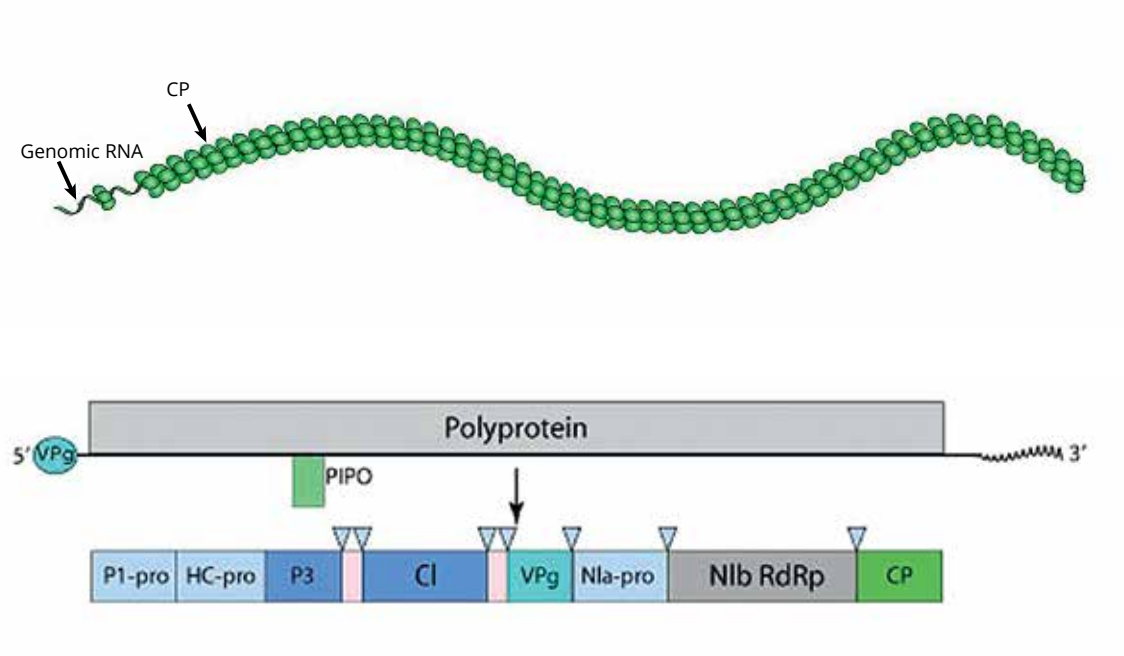

FIGURE 3: structure of the potato Y virus (PVY).

Conventional genetic improvement is an efficient way to introduce resistance to the virus. It consists in breeding individuals of the same species that display different characteristics to later select those that are born with the traits the breeder is looking for. In potatoes, this is a slow and complicated procedure, due to the genetic characteristics of the species. Besides, the Spunta variety (more than 90% of the production for fresh consumption in Argentina) produces very few botanical seeds, which makes breeding even more difficult.

Resistance to the virus through genetic engineering

The biotechnological approaches are more effective to introduce resistance to plant viruses. The technology to do that has been present since 1986 and has been permanently improving since then. In Argentina, the potato genetic transformation has been present since 1989-90 in the INGEBI-CONICET (Institute of Research in Genetic Engineering and Molecular Biology).

In 1999, the CONICET signed an agreement with our firm to develop the Spunta potato plants, resistant to PVY.

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Resistance mechanism

The plants have an ample range of defense mechanisms against vegetable pathogens, but they do not have an immune system like that of animals, based on antibodies.

In the case of plant viruses, the defense mechanism is highly selective and it is based on the detection of the presence of the NDA or RNA of the invading virus to eliminate it. Each plant cell has that mechanism.

When the virus enters the plant, a fight between the virus and the defense mechanisms starts. Depending on who the winner is, the plant can build up high resistance or immunity. If the pathogen wins this fight, the plant will get sick.

The applied technology in our case consists in establishing this defense mechanism on a permanent basis directing resistance against the PVY virus. This process is similar to the one a vaccine produces in humans and animals, through which the immune system activates and gets ready to fight against a specific pathogen.

The Post-Transcriptional Gene Silencing (PTGS) is a change introduced through genetic engineering in Spunta TICAR. It only affects the regulation of a defense mechanism typical of the plant and does not introduce new characteristics to it other than the resistance to this virus. It is a process that allows the degradation of a specific RNA, in this case, the RNA of the PVY virus.

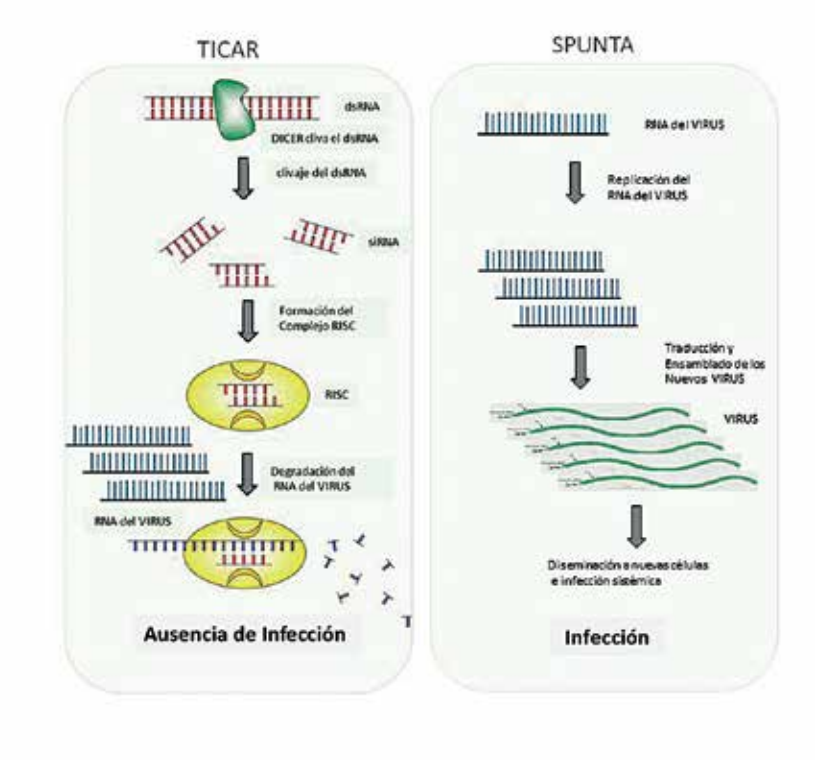

Figure 4: the Spunta defense system (right) vs. the TICAR defense mechanism (left)

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The Post-Transcriptional Gene Silencing, a natural defense mechanism of plants against viruses

As FIGURE 4 shows (left), when the PVY virus infects a TICAR plant, it faces an already-established defense mechanism, since the plant produces double-stranded RNA (dsRNA) that is similar to the sequence of the virus and is fragmented by the DICER enzyne into small pieces called siRNAs. These guide a degradation complex (RISC) to eliminate the RNA that is similar to these siRNAs, that is to say the PVY virus. Instead, as it is observed in FIGURE 4 (right), when the PVY infects a Spunta potato plant, the virus or its RNA can enter a cell and multiply to give rise to new viruses. Consequently, the plant will get sick as it has failed to make the defense mechanism work at the right moment (PTGS).

This mechanism has been used to get papaya plants resistant to PRSV. These plants have been cultivated and marketed since 1998 in Hawaii, and they have also obtained approval in Canada and Japan. China has also produced and marketed PRSV-resistant papaya since 2006. Recently, the same mechanism has been used to obtain non-browning apples and potatoes. Browning is prevented thanks to the silencing of the enzyme called polyphenol oxidase. These events have obtained approval to be sold in the United States and Canada. The EMBRAPA in Brazil has also developed bean plants resistant to BGMV and their commercialization has been approved.

Simplot Plant Sciences, the second most important industry of frozen pre-fried sticks around the world, uses **Papa Spunta TICAR**'s technology and its "Innate" potato has obtained approval in key foreign markets, including Japan. It has some applications for foreign-market access pending. Simplot obtained approval to sell its first-generation Russet Burbank in Japan last August, 2017. The Innate first-generation Burbanks, Atlantics and Ranger Russet -all bred to resist bruising, to avoid turning brown after cutting and to possess low levels of acrylamide, which is a potentially harmful chemical created during frying of some starchy foods — were approved last spring in Australia and New Zealand. The company has also applied for first-generation approval in China, South Korea, Taiwan, Malaysia, Singapore and Mexico, and plans to apply in the Philippines soon. Simplot has already received approval for second generation Innate varieties in both Canada and the U.S.

In the United States, Simplot has recently sold out its inventory of Innate fresh potatoes, marketed under the White Russet label, for a third consecutive year. Innate has been carried in roughly 4,000 supermarkets in 40 states. Retailers and food service professionals have become willing to pay a premium for Innate potatoes because they avoid bruising, even late in the season when the problem is rampant.

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Spunta TICAR. **Development, field trials, environmental and food safety**

Once the expected results were achieved in the laboratory, a great variety of potentially PVY-resistant events (more than 400) were cloned in vitro to obtain microplantules. The seedlings were acclimated and challenge tests with the virus were done in greenhouses (295 of those clones were discarded) and then on the field (other 83 clones were discarded at this stage). This allowed the selection of those clones that better reacted against the disease. Then, new field experiments were conducted to value yield levels and behavior under production conditions.

These were carried out under the strict control of the National Department of Agricultural Biotechnology from the ex-National Ministry of Agroindustry and were especially and permanently monitored by the National Advisory Board on Agricultural Biotechnology, CONABIA.

INGEBI-CONICET and Tecnoplant carried out field trials that allowed them to select two promising events that were resistant to the virus and to move forward towards the commercial launching of the product.

Several selections in greenhouses and nine field trials in four different spots in the country between 1998 and 2001 (see FIGURE 5) gradually allowed the experts to choose the most promising clones until they finally reached the TIC-AR 233-5, which was selected to move to Phase II of CONABIA. At this stage, important demands and regulations had to be met so as to guarantee the environmental biosafety of the event.

The chosen event was resistant to PVY in tests in which 85% of the Spunta control plants were infected. All the trials were authorized and approved by the CONABIA. Virus resistance was confirmed in several post-tests in which there

FIGURE 5 Comparative yield test with two of the most promising events

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was absence of infection in the TIC-AR plants. The Spunta control plants, on the other hand, showed different degrees of infection.

Concurrently, the TIC-AR 233-5 variety underwent consumption suitability tests. Different phenotypical and biochemical characteristics that demonstrated that the composition and nutritional value of the TIC-AR 233-5 are identical to those of the original Spunta variety were analyzed.

To increase the environmental safety levels of our **Spunta TICAR** event, field gene flow assessment was carried out through which the absence of horizontal transfer to related species and to other potato varieties was confirmed.

All this information was published in Transgenic Research, in the article Field testing, gene flow assessment and pre-commercial studies on transgenic Solanum tuberosum spp. Tuberosum (cv. Spunta) selected for PVY resistance in Argentina by Bravo Amonacid et al.

Besides, the genome of the TIC-AR 233-5 variety was completely sequenced. That genomic sequence was compared to the potato reference genome. Thus, the modifications and the fact that the unwanted changes had not taken place were confirmed. Besides, the place where the insertion occurred was verified.

During 2014, the Department of Agricultural Markets of the ex-Ministry of Agroindustry of the Nation made a positive report on the impact of the product on the market. Besides, with the new national government in 2017, this was evaluated again and the conclusions were the same as those in 2014.

On April 29, 2015, the CONABIA approves the TICAR transgenic event, fulfilling with the last of its demanding requirements.

In October, 2015, Decree 399/2015 of the ex-Ministry of Agriculture, Farming and Fishing of the Nation, through which the commercialization of the TICAR transgenic potato was approved, was published in the Official Bulletin of the Argentine Republic. This was subject to the presentation of additional information the Health and Agri-food Quality National Service, SENASA, could require. The information that guaranteed the innocuousness of the event for consumption was supplied and, by July 2018, the SENASA approved its commercialization.

On August 8, 2018, the ex-Ministry of Agroindustry of the Nation, by Decree 65/2018, frees the commercialization of this transgenic product, a potato resistant to PVY virus.

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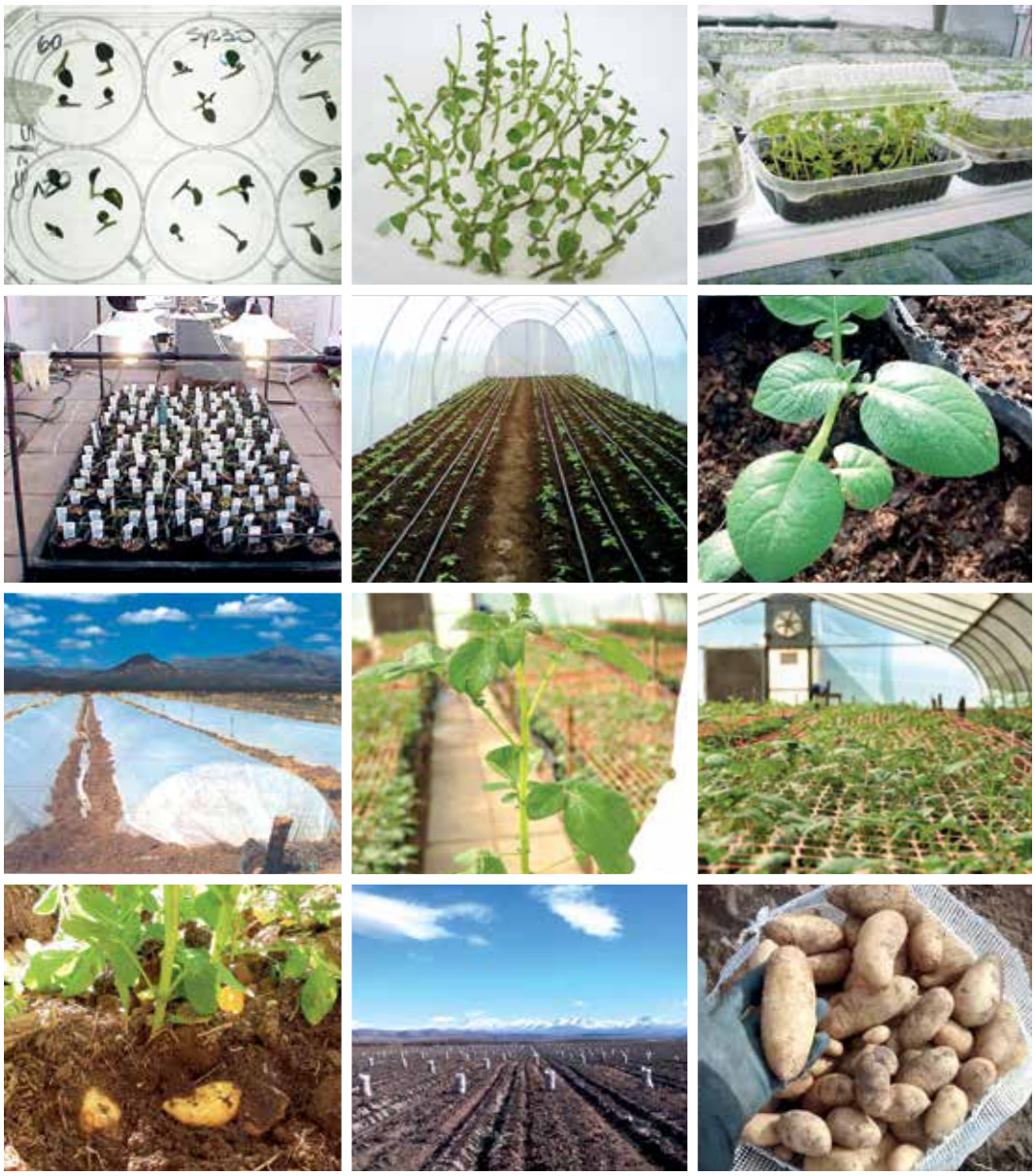

Impact of Spunta Ticar commercialization in the country

- Lower production costs for consumption of the fresh product per campaign (between 7 and 9%).
- Economic improvement of about 40 and 45 million dollars per year.
- Better performance and higher productivity.
- Higher profitability for the producer.
- More competitiveness of the value chain.
- Broader geographical borders for the production of the potato seed in Argentina.
- Less agrochemical use, especially pesticides, which leads to less environmental impact.
- Potential to generate new related events of interest for potato cultivation.
- Potential to export technology.

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TECNOPLANT S.A.

- We are a completely Argentine capital firm.
- We are a small- and medium-sized company that is part of a pharmaceutical group (SIDUS).
- The transgenic potato was developed in Argentina by Argentine researchers, producers and businessmen.
- They have been working since 1999.
- This new potato variety is the product of a joint public/private venture between Tecnoplant and INGEBI-CONICET.
- The project has been funded by the Sidus Group.
- Its implementation improves production at national level.
- This technology could be exported.
- The fact that a national firm from a peripheral country achieves a vegetable transgenic event with impact on the value chain is unprecedented.
- The firm aims to generate a model to recover the investment through royalties that respect the needs of the producer and that are charged on the seed that is purchased and/or put aside by the producer.
- We have generated an innovative model to pay intellectual property rights.

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Achievements of the SIDUS GROUP in the argentine biotechnological sector

- 1° Recombinant pharmaceutical produced in cells (EPO)
- 1° Recombinant pharmaceutical produced in bacteria (FILLGASTRIM)
- 1° Cow cloning
- 1° Horse cloning
- 1° Transgenic cow producer of HG
- 1° Transgenic cow producer of bovine HG
- 1° Transgenic cow producer of insulin
- 1° Transgenic event developed in Argentina (Pampa cows, producers of HG)
- 1° Vegetable transgenic event developed in Argentina by an Argentine firm (Spunta TICAR)
- 1° Pharmaceutical based on lyophilized blueberries

Marie Orensanz, argentine artist

"Implementing innovations is difficult and constitutes a peculiar economic function. First, because it is outside the routine tasks that everyone understands; and secondly, because the outside world presents a multiform resistance to this. To act with confidence, skills are required that only occur in a small fraction of the population and characterize both the type and the role of the entrepreneur. This funciphn does not consist essentially in inventing something or in creating in another way the concidions that the company exploits, it consists in achieving accomplishments."

(Joseph Schumpeter, american economist)

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